# MUTANTS OF *ESCHERICHIA COLI* K 12 ALTERED IN THEIR ABILITY TO STORE GLYCOGEN

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It was shown previously (Sigal *et al.*, 1964 ; Segel *et al.*, 1965) that a branched polysaccharide similar to animal glycogen is accumulated by certain bacteria when growth is limited by some factor other than carbon source.

Three enzymes are necessary for glycogen accumulation :

1) The adenosine-diphosphate-glucose : α-4-glucosyl transferase (glycogen synthetase) which transfers glucose from adenosine-diphosphate-glucose to an α-1,4-glucan primer (Greenberg and Preiss, 1964 ; Shen *et al.*, 1964 ; Greenberg and Preiss, 1965 ; Preiss and Greenberg, 1965).

2) The adenosine-diphosphate-glucose-pyrophosphorylase catalyzing the synthesis of adenosine-diphosphate-glucose from adenosine-triphosphate (ATP) and α-glucose-1-phosphate. The presence of this enzyme has been reported in extracts of several bacteria (Shen and Preiss, 1964; Shen and Preiss, 1965).

3) The branching enzyme (α-1,4-glucan : α-1,4-glucan 6-glycosyltransferase, EC 2.4.1.18) necessary for the formation of the branch chains in the glycogen molecule. This enzyme has been detected in *E. coli* (Sigal *et al.*, 1965) and in *Arthrobacter globiformis* (Zevenhuizen, 1964)

All these enzymes have been shown to be present in log phase cells which however accumulate little or no glycogen (N. Sigal, 1968). The mechanism by which glycogen synthesis is triggered as soon as the growth rate is reduced is still unknown.

The present work reports the isolation and the biochemical study of different mutants of the glycogen system.

Mutations have been induced by N-methyl-N'-nitro-N-nitrosogua-

nidine (100 µg/ml) treatment as described by Adelberg and collaborators (1965) on the $F^-$ strain PA 601 ($thr^-$, $leu^-$, $pro^-$, $ad^-$, $his^-$, $arg^-$, $thi^-$).

The detection of mutants was made by flooding colonies with iodine solution (0.2 $I_2$ in 0.4 % KI) on solid media. Colonies of the parent strain which accumulate glycogen on synthetic medium containing 80 mg of ammonium chloride and 3 g of glucose per liter are red-brown with the iodine solution.

We observed three other types of colonies :

- colorless (A)
- blue (B)
- dark brown (C)

These three types of colonies seemed to correspond to three types of mutants differing in their ability to accumulate glycogen. Three colonies of each type were analyzed.

- Colorless mutants (A) do not accumulate glycogen during the stationnary phase of growth. Cells of the parent strain, cultured in synthetic medium containing a limiting amount of threonine and excesses of the other factors, accumulate glycogen to over 20 % of the cell dry weight when threonine is depleted. The cells of these mutants seem unable to synthesize glycogen because they lack glycogen synthetase (table). The activity of this enzyme is practically undetectable. The levels of the ADPG-pyrophosphorylase are similar to or higher than that of PA 601 strain.

- During the stationnary phase, the mutants whose colonies stain blue with iodine solution (B) accumulate small amounts (4-5 % of dry weight) of a polyglucose which is not very soluble in water but soluble in sodium hydroxide. This compound has been extracted from bacteria, purified and characterized by methods previously described (Segel *et al.*, 1965). It has the following properties : the λ max. of the iodine complex is at 580-590 mµ ; the β-amylolysis limit is 85 %. This value is that of a practically not-branched polysaccharide. The degree of polymerisation of this polyglucose may be estimated from its λ max. as roughly 55-61 glucose units (Bailey and Whelan, 1961). In the three B mutants the glycogen synthetase level is about 10 % that found in the parent strain. One of them (table) has an ADPG-pyrophosphorylase level a third that of the PA 601 strain but the two others have the same activity as the parent strain.

Table

SYNTHESIS OF POLYGLUCOSE AND ENZYMES BY MUTANTS AND PARENT STRAIN

| Strain | Iodine coloration | Polyglucose % dry weight | Specific activities of glycogen synthetase a | Specific activities of ADPG-pyrophosphorylase b |
|---|---|---|---|---|
| PA 601 (parent strain) | red-brown | 17.3 | 1.185 | 0.500 |
| Mutant A | colorless | 2.3 | ∿.0 | ∿ 0.770 |
| Mutant B | blue | 4.95 | 0.133 | 0.135 |
| Mutant C | dark brown | 44.5 | 0.84 | 0.495 |

Enzymes were determined on sonicates of the cells which had been grown in synthetic medium limited by threonine.

a) expressed as µmoles of glucose $^{14}C$ incorporated into glycogen per mg of protein in 15 minutes.

b) expressed as µmoles of ATP-$^{32}P$ formed per mg of protein in 10 minutes.

- The C mutants accumulate glycogen in amounts frequently exceeding 40 % of the cell dry weight during the stationnary phase. However the levels of glycogen synthetase and ADPG-pyrophosphorylase are quite similar to those of the enzymes of the parent strain (table); the level of branching enzyme is normal.

The polyglucose accumulated by this mutant has been extracted and characterized. It has the following properties : the $\lambda$ max. of the iodine-complex is 480 m$\mu$ ; the average chain length ($\overline{CL}$) determined by periodate oxidation and $\alpha$- and $\beta$-amylase degradation is 12 units. These characteristics correspond to those of animal and bacterial glycogen. The dark brown coloration of colonies of these mutants does not seem to correspond to a different structure of the polyglucose accumulated but corresponds to a higher content of glycogen. It is interesting to note that the cells of this dark-brown mutant seem to accumulate glycogen even during the log phase of growth and that the accumulation rate, during the stationnary phase, is higher in this mutant than in the parent strain.

Discussion :

It is highly probable that the A mutants have lost the structural gene for glycogen synthetase. The lack of this enzyme prevents any synthesis of polysaccharide in spite of the presence of ADPG-pyrophosphorylase. In the blue mutants (B) the polyglucose is linear. This fact seems to indicate the lack of branching enzyme. Branching activity is usually detected in the particulate fraction of extracts (sedimented at 105,000 x g) after separation from an $\alpha$-amylase (Sigal, 1968) ; in the B mutants branching activity is not found in this fraction. The low accumulation of the blue polysaccharide could be due to the reduced activity of glycogen synthetase or to the presence of some degradative enzymes.

The type C mutation does not seem to modify the levels of the enzymes of the glycogen system and the polyglucose accumulated is not different from that of the parent strain. Since the levels of the enzymes in these mutants are similar to those of the parent strain, a mechanism of derepression seems very improbable. It is thus reasonnable to postulate that there is a genetically controled inhibition of glycogen synthesis during log phase, in the parent strain, and that this inhibition is removed by the C mutation.

The genes of the glycogen system have been mapped. Details will be published elsewhere.

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While this paper was being prepared a preliminary report by Govous, Sabraw and Preiss appeared in the Federation Proceedings, *27*, 289 (1968). This report describes two mutants similar to ours.

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